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Chemotherapy and Biochemistry of <u>Leishmania</u>

Final Report

LINDA L. NOLAN, Ph.D.

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UNIVERSITY OF MASSACHUSETTS
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▲The overall aim of this research for the last five years has been to determine the mode of action of promising antileishmanial agents for the purpose of rational drug development. Enzymatic differences and requirements between the synthesis of nucleic acids in the parasite and host were studied for the purpose of chemotherapeutic exploitation. A comparison of the enzymes of the pathogenic protozoa to those of man is of fundamental importance to the search for much needed chemotherapeutic agents. Nucleic acid metabolism in trypanosomatids is unqiue in several ways: (1) they lack the ability to synthesize purines de novo, depending entirely on the salvage pathway for their supply of purine nucleotides; (2) many of the enzymes involved in nucleic acid biosynthesis either have unusual substrate specificities or unusual subcellular localizations; (3) a large proportion of the DNA which is produced is incorporated into a unique organelle known as the kinetoplast; and (4) the DNA polymerase isolated from these organisms demonstrates major differences from its mammalian counterpart.   (over) ──►

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19. continued.

There is very little information concerning the DNA and RNA polymerases of
Leishmania spp.

Our aim has been the isolation and characterization of the DNA and RNA polymerases
of Leishmania mexicana and search in vivo and in vitro for inhibitors of these
enzymes for chemotherapeutic exploitation.

Sinefungin has been found to be a potent antiparasitic agent at levels which are
non-toxic to mammalian cells. Our laboratory has found that it drastically
affects DNA synthesis in Leishmania spp. We are currently investigating its
exact mode of action, to aid in rational drug development.

We have continued our studies on the mode of action of Formycin B, an anti-
leishmanial purine analog, and have shown that it is converted to Formycin A and
preferentially incorporated into mRNA as opposed to tRNA and rRNA.

A-1

TABLE OF CONTENTS

1

RESUME OF PROGRESS

Culture Methods:

The organisms used in this project have been obtained from Walter Reed Army Institute of Research through the courtesy of Dr. Joan Decker-Jackson and Dr. Jonathan Berman. The organisms used most have been *Leishmania mexicana amazonensis* WR 227 and *L. donovani* WR 130 (Khartoum strain-drug sensitive visceral leishmaniasis). Other organisms presently being cultivated in this laboratory are *L. braziliensis* WR 424 (Murray isolate from Panama causing cutaneous leishmaniasis), *L. braziliensis* WR 063 (Terborgh isolate from Peru, causing mucocutaneous leishmaniasis). These organisms are maintained by weekly transfers into Schneider's medium [Grand Island Biological Co., Grand Island, N.Y. (Gibco)] containing 10% heat inactivated fetal bovine serum (HIFBS: GIBCO).

For growing large batches of leishmaniae promastigotes, Brain Heart Infusion Medium (BHI) containing 37 g Difco Brain Heart Infusion/liter water, 10% heat inactivated serum and 26 µg hemin/ml was used. Cells were grown at 26° in 2000 ml wide Fernback flasks containing 250 ml of BHI and harvested during the exponential growth phase (about day 4).

For defined biochemical experiments the medium of Steiger and Black was used. This medium was used for all transport, uptake and reversal experiments. The cells were depleted of purines by transferring an inoculum from Brain Heart Infusion into Steiger and Black medium with purine omitted, but with 5% heat inactivated fetal bovine serum. The cells were incubated in this medium for 48 hr at 26°C. The cells were then aseptically centrifuged at 5000x g for 10 min and resuspended to the desired number into fresh Steiger and Black medium Minus purine. By treating the cells in this manner, we avoided as much as possible interference of the metabolism of the compound being tested by the purines in the medium.

RE 1X (Steiger and Black) --- Components per liter:

A)
8.0	g	NaCL
400	mg	KCL
200	mg	$MgSO_4.7H_2O$
60	mg	$Na_2HPO_4.2H_2O$
60	mg	KH_2PO_4
2.0	g	glucose

C)
300	mg	L-glutamine
1.0	g	$NaHCO_3$
14.25	g	HEPES (=60 mM)
20	mg	adenosine

B)
200	mg	L-arginine
100	mg	L-histidine
100	mg	L-iosleucine
300	mg	L-leucine
250	mg	L-lysine.HCL
50	mg	L-methionine
100	mg	L-phenylalanine
300	mg	L-proline
400	mg	L-threonine
50	mg	L-tryptophan
50	mg	L-tyrosine
100	mg	L-valine

D)
1	mg	D-biotin
1	mg	choline chloride
1	mg	folic acid
2	mg	i-inositol
1	mg	niacinamide
1	mg	D-pantothenic acid) (hemi-calcium salt)
1	mg	pyridoxal.HCL
0.1	mg	riboflavine
1	mg	thiamine.HCL

E)
2.5	mg	haemin

This growth test model, developed in our laboratory, has enabled us to evaluate *in vitro* many newly synthesized test compounds rapidly. Promising compounds were then evaluated further by testing them using the scheme in Fig. 1 on many of the purine metabolic enzymes we have isolated in our laboratory. These enzymes are shown in Fig. 2 and Table 1. This approach has enabled us to evaluate *in vitro* and *in vivo* many test compounds rapidly, pinpointing the biochemical basis of activity (or lack of it) against *Leishmania* and *Trypanosoma* species. This information has been used to guide drug synthetic schemes, and to substantially reduce the number of compounds sent to the *in vivo* efficacy screen directed by WRAIR.

2

Figure 1

IN VITRO ANTILEISHMANIAL DRUG EFFICACY & MODE OF ACTION
L. Nolan: "Chemotherapy and Biochemistry of Leishmaniases"

PROMASTIGOTE
GROWTH INHIBITION

NONE
No Further Testing

YES
PROTEIN?/RNA?/DNA?
Radioactive precursor:
^{14}C-phenylalanine/-uridine/-thymidine
Transport & Incorporation in Leishmania

PROTEIN INHIBITION
1. in vitro protein translation
   from Leishmania messenger RNA
   (mRNA)
2. protein carboxymethyl
   transferase inhibition

RNA INHIBITION
1. Incorporation of test drug into RNA (via mass spec: assay of total RNA, 'tRNA'; ribosomal RNA, 'rRNA'; & mRNA)
2. Assay for inhibition of parasite RNA polymerase III, a DNA-dependent RNA polymerase, first isolated and purified by Dr. Nolan (Mol. Cell. Biol. 1986. In Press).
3. Inhibition of mRNA methyltransferase.

DNA INHIBITION
Assay for drug inhibition of following 17+*
Leishmania enzymes isolated, purified, with
all assay parameters optimized by Dr. Nolan:

1. DNA polymerase-alpha (manuscript In Press, 1986),
2. S-adenosylmethionine synthetase,
3. S-adenosylmethionine decarboxylase,
4. adenine phosphoribosyltransferase,
5. S-adenosylhomocysteine hydrolase,
6. hypoxanthine-guanine phosphoribosyltransferase,
7. guanine 7-methyltransferase,
8. xanthine phosphoribosyltransferase,
9. adenylate deaminase,
10. adenylate synthetase.

11. DNA methylase
12. nucleosidases
13. adenine deaminase
14. DNA methylase
15. guanine deaminase
16. *nuceosidases,
17. OTHER: phospholipid methylation (resulting in loss of membrane fluidity with receptor function loss).

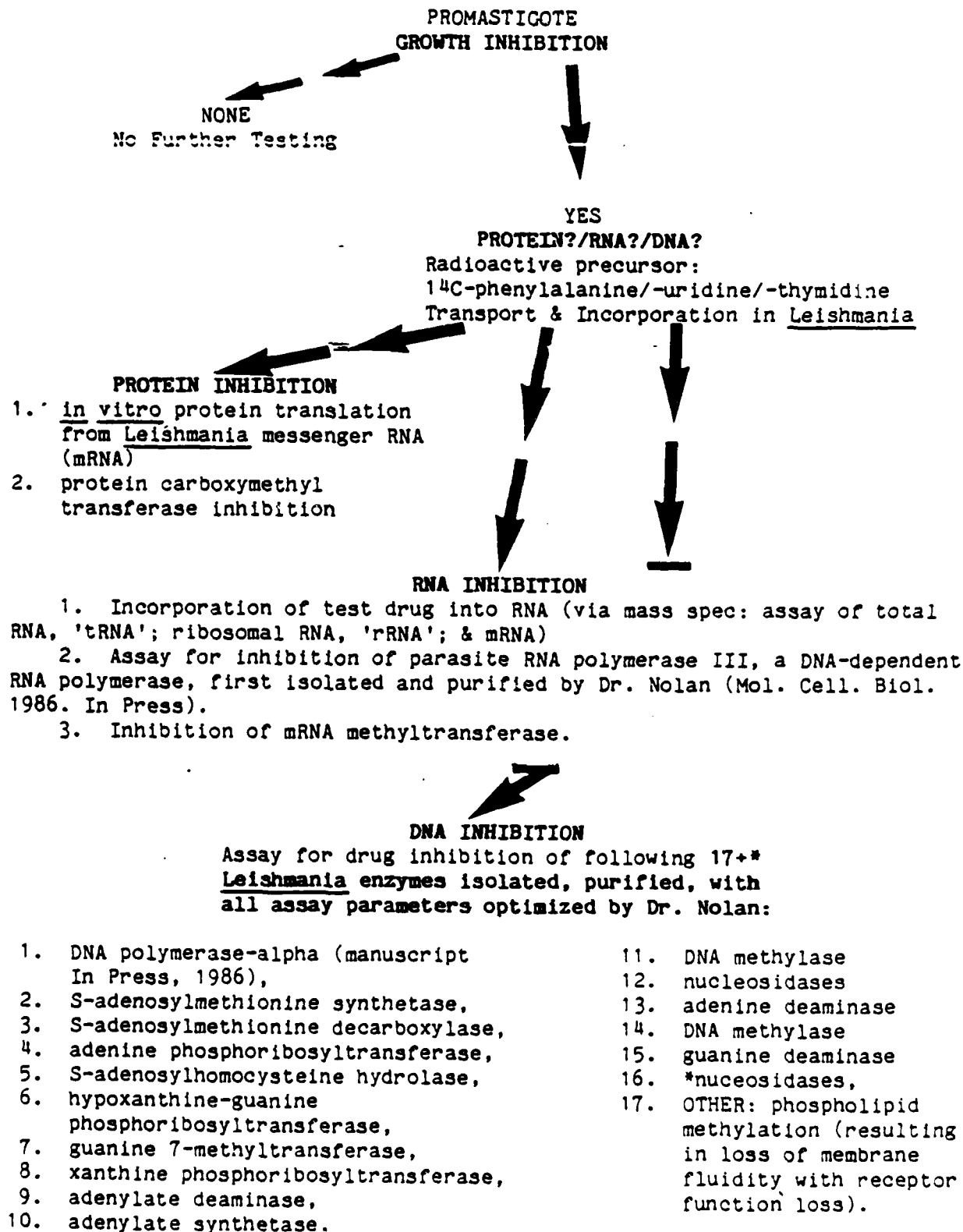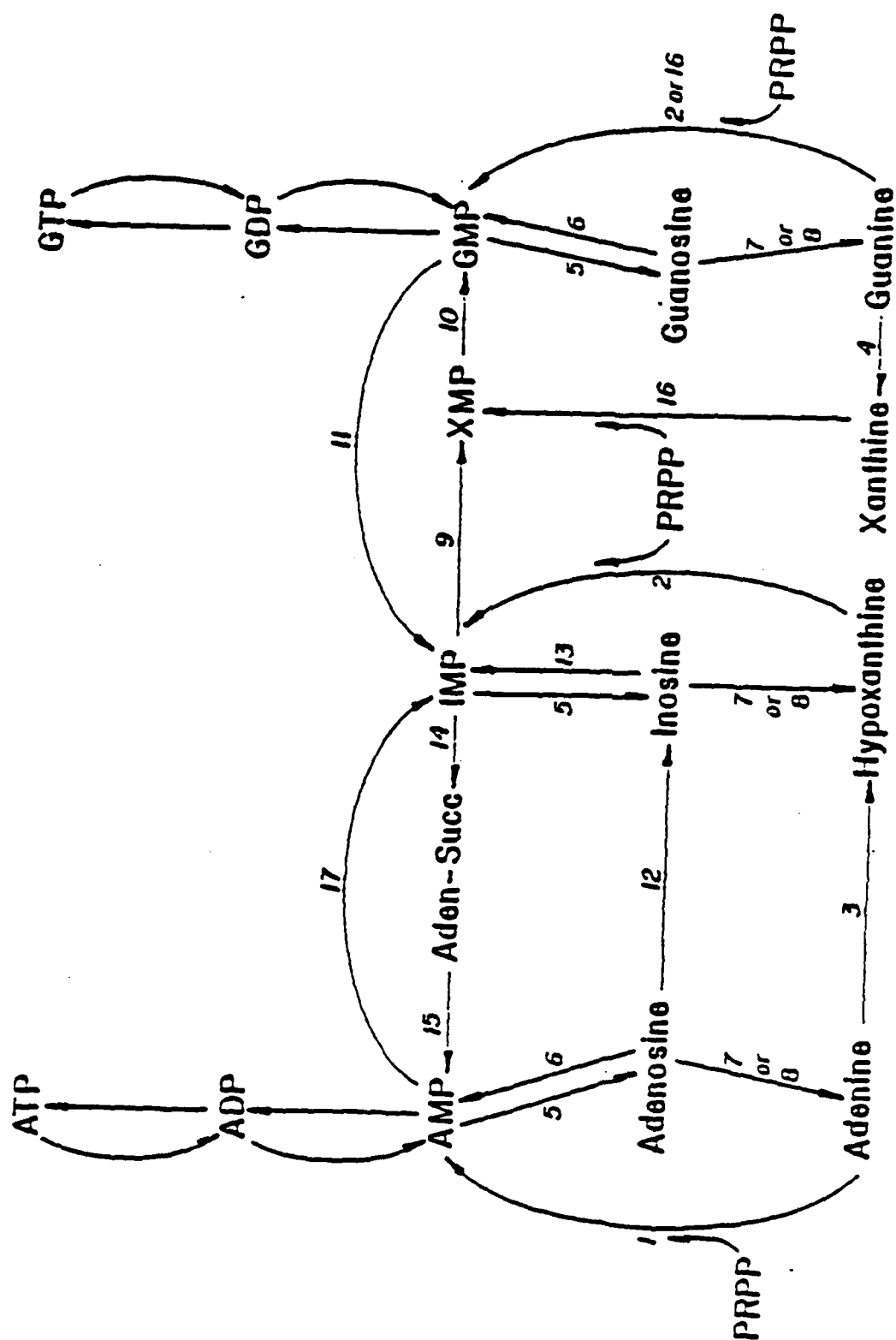Figure 2

Interconversion of Purines

Table 1. Some of the reactions involved in interconversions of purines and purine derivatives. This is a compendium of reactions present in various organisms.

Enzymes: 1, adenine phosphoribosyltransferase (EC 2.4.2.7); 2, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); 3, adenine deaminase (EC 3.5.4.2); 4, guanine deaminase (EC 3.5.4.3); 5, 5'-nucleotidase (EC 3.1.3.5); 6, adenosine kinase (EC 2.7.1.20); 7, purine nucleoside hydrolase (EC 3.2.2.1); 8, purine nucleoside phosphorylase (EC 2.4.2.1); 9, IMP dehydrogenase (EC 1.2.1.14); 10, GMP synthetase (EC 6.3.4.1); 11, GMP reductase (EC 1.6.6.8); 12, adenosine deaminase (EC 3.5.4.4); 13, inosine kinase (EC 2.7.1.73); 14, adenylosuccinate synthetase (EC 6.3.4.4); 15, adenylosuccinate lyase (EC 4.3.2.2); 16, guanine phosphoribosyltransferase (bacterial); 17, AMP deaminase (EC 3.5.4.6).

Abbreviations: Ad, adenine; Hx, hypoxanthine; X, xanthine; Gu, guanine; AdR, adenosine; In, inosine; GuR, guanosine; 8-AMP, adenylosuccinate; AMP, adenosine 5'-monophosphate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; IMP, inosine 5'-monophosphate; XMP, xanthosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; GDP, guanosine 5'-diphosphate; GTP, guanosine 5'-triphosphate.

5

·By using the above test systems, we can determine the mode of action of the compound so that this information can be used to (1) synthesize better derivitives (2) explain possible host toxicity and lead to protocols to avoid this complication (3) help understand and combat resistance to the compound.

Compounds (sent by WRAIR) Tested

1) BK 63863 Thiopurinol riboside
2) BK 74731 Oxoformycin
3) BK 86124 Allopurinol riboside
4) BK 86133 5-Azaxanthosine
5) BK 86142 7-Ribosyl-3-deazoguanine
6) BK 63005 3-β-D-Ribofurano sylpyrazolo-[4,3-d] pyrimidine-7-thione
7) BK 48464 6-Aminoallopurinol Riboside
8) BK 95141 3-Ethoxy-6-methylthio-1-β-D-ribofurano sylpyrazolo-[3,4-d] pyrimidine-4(5H)-one
9) BK 95169 3-Ethoxy-1-β-D-ribofuranosylpyrazolo-[3,4-d] pyrimidine-4(5H)-one
10) BK 95187 6-Methylthio-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo-[3,4-d] pyrimidine-3-carboxamide
11) BK 95203 7-Amino-5-chloro-3-β-D-ribofuranosyl-pyrazolo-[4,3-d] pyrimidine (5-chloroformycin)
12) BK 95730 6-Aminoimidazo[4,5-C] pyridin-4(5H)-one (3-Deazaguanine)

Compounds Which Were Significantly Inhibitory at the End of 96 Hrs

Organism	Concentration of BK 63005 (% Inhibition)		
	10 µM	100 µM	500 µM
L. donovani 130	74	82.4	83.8
L. mexicana 227	68.4	83.5	83.0
L. braziliensis 424	34.6	52.9	52.9

Organism	Concentration of BK 74731 (% Inhibition)		
	10 µM	100 µM	500 µM
L. donovani 130	31.8	78.7	84.9
L. mexicana 227	15.5	71.6	83.5
L. braziliensis 424	20.7	36.0	42.2

Organism	Concentration of BK 63863 (% Inhibition)		
	10 µM	100 µM	500 µM
L. donovani 130	60.6	74.3	70.1
L. mexicana 227	58.3	71.9	75.4
L. braziliensis 424	3.1	17.6	24.3

Organism	Concentration of BK 86124 (% Inhibition)		
	10 µM	100 µM	500 µM
L. donovani 130	46.1	50.1	48.7
L. mexicana 227	30.4	41.1	67.9
L. braziliensis 424	1.8	5.8	8.7

Organism	Concentration of BK 48464 (% Inhibition)		
	10 uM	100 uM	500 uM
L. donovani 130	9.8	35.2	56.2
L. mexicana 227	—	13.8	20.4
L. braziliensis 424	—	27.9	41.3

Compounds Which Showed Up To 20% Stimulation

1. BK 86133 5-Azaxanthosine

2. BK 86142 7-Ribosyl-3-Deazoguanine

These compounds appear to be broken down and metabolized as natural purines.

Figures 3-7, show the response of different Leishmania spp. to the most inhibitory purine analogs (sent by WRAIR).

Compounds which were inhibitory were tested for reversal of inhibition by natural purines. The concentration of the analog was 50µM and that of the natural purine was 200 µM. Table 2 shows the ability of natural purines to reverse the inhibition by the analogs. The purines which were most effective in reversing inhibition by the particular analog are as follows:

Purine Anolog	Purine Most Effective in Reversing Inhibition
3-B-D-Ribofurano-sylpyrazolo-[4,3-d] pyrimidine-7-thione BK 63005	Inosine
Oxyformycin BK 74731	Adenosine
Thiopurinol riboside BK 63863	Guanosine (not very effective)
Allopurinol riboside BK 86124	Adenosine
6-Aminoallopurinol riboside BK 48464	Adenosine, Hypoxanthine (equal)
6-Aminoimidazo[4,5-C] pyridin -4(5H)-one (3-deazaguanine) BK95730	Guanosine

Other purine anologs (not sent by WRAIR) have been shown to be very potent growth inhibitors of promastigotes of L. mexicana #227. The following table compares the toxicity of these compounds to some of the most promising anologs sent by WRAIR.
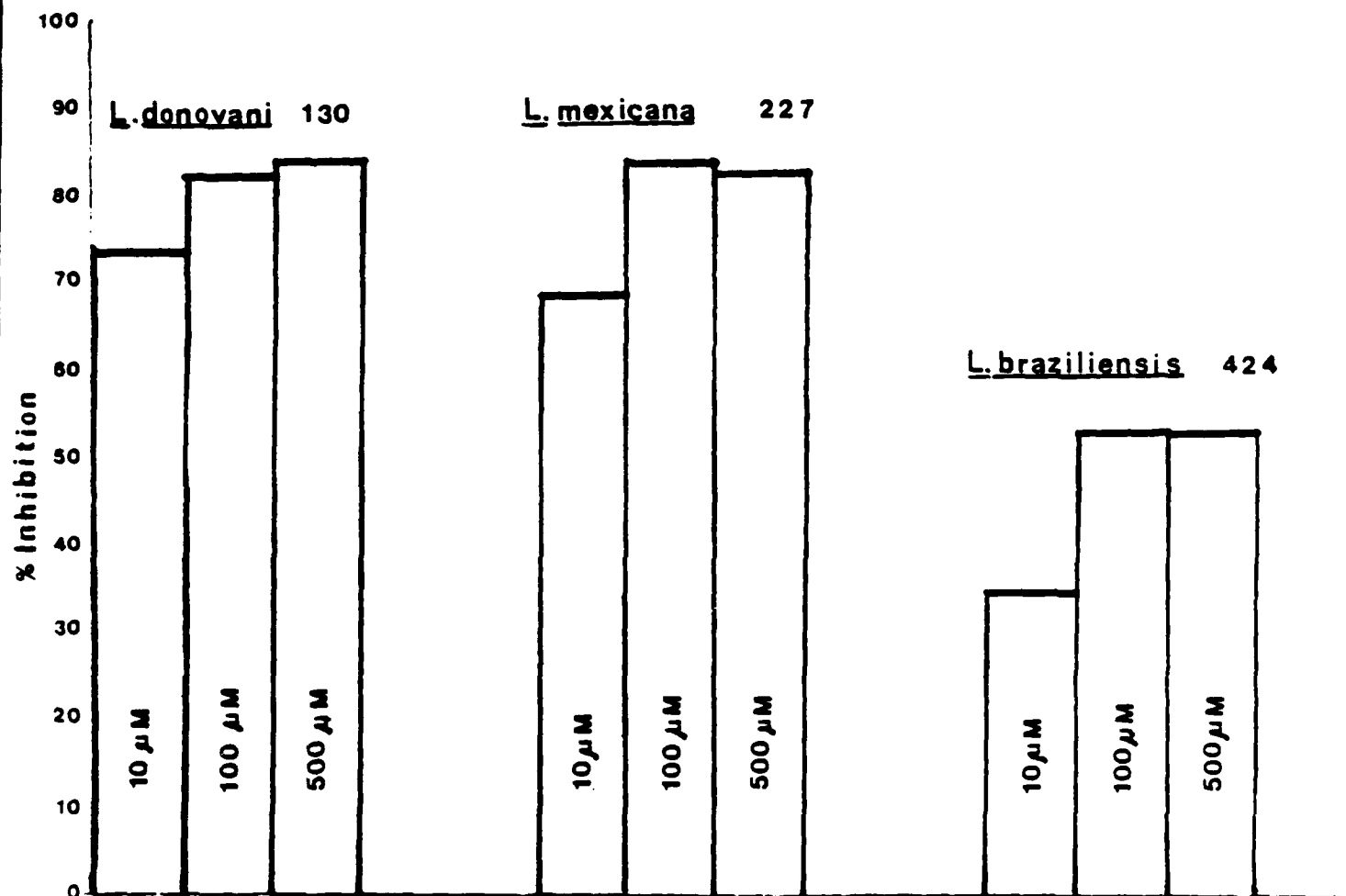
Fig. 3

3-β-Ribofuranosylpyrazolo [4,3-d̲]-pyrimidin-7-thione

Drug BK63005

Fig. 4

Oxyformycin

Drug BK74731

% Inhibition plot for Drug BK74731 showing L. donovani 130, L. mexicana 227, and L. braziliensis 424 at 10μM, 100μM, and 500μM.

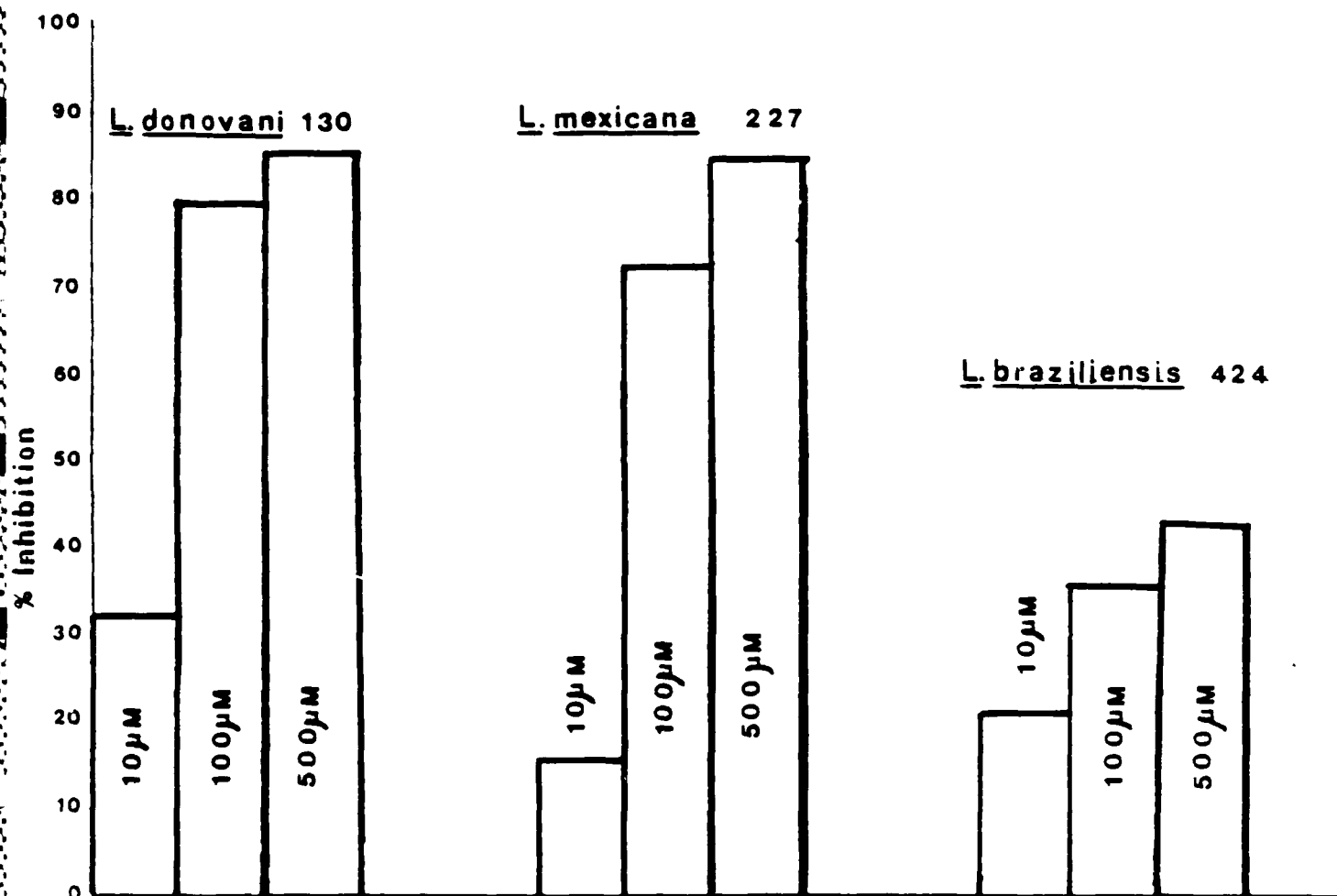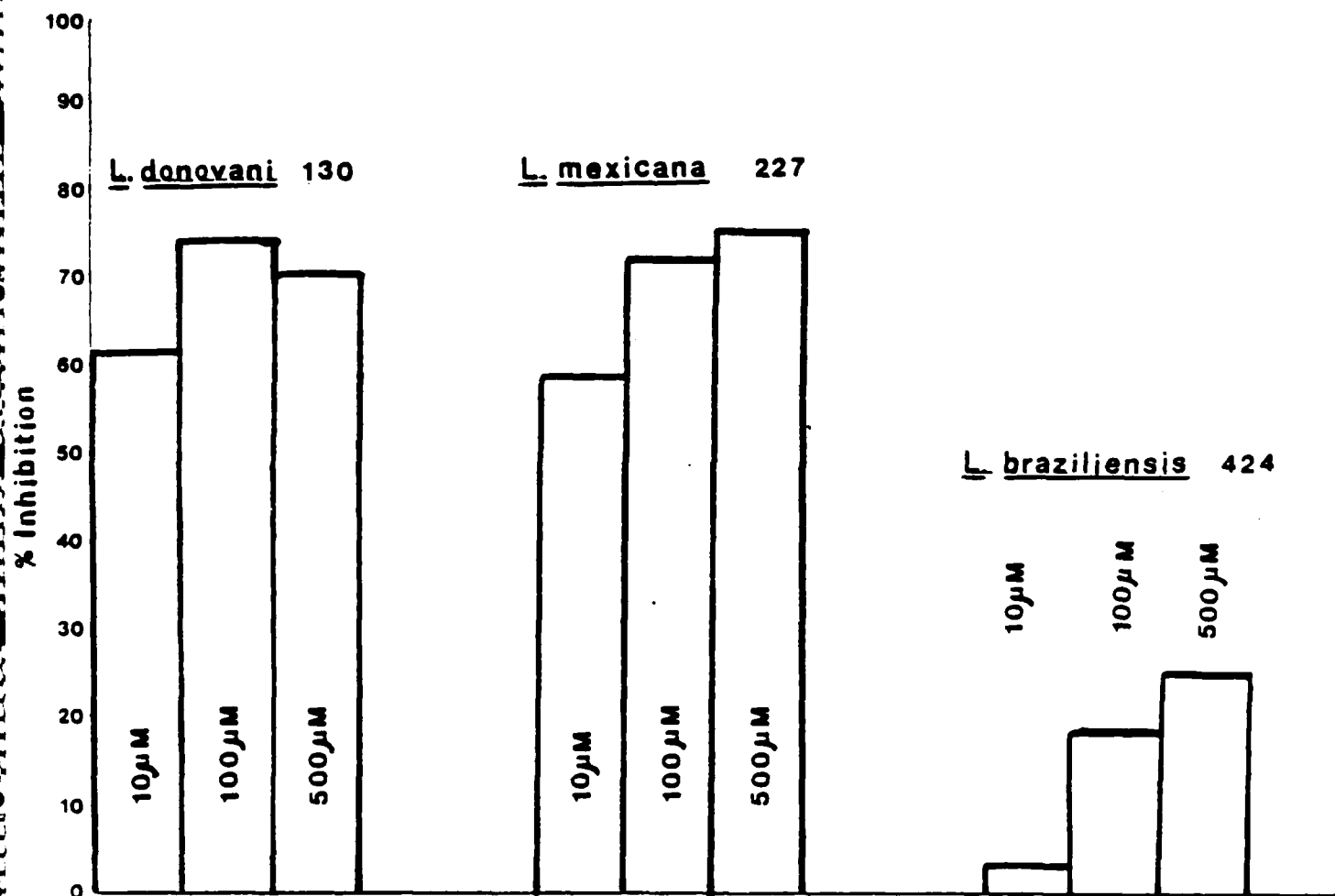Fig. 5

Thiopurinol riboside

Drug BK63863
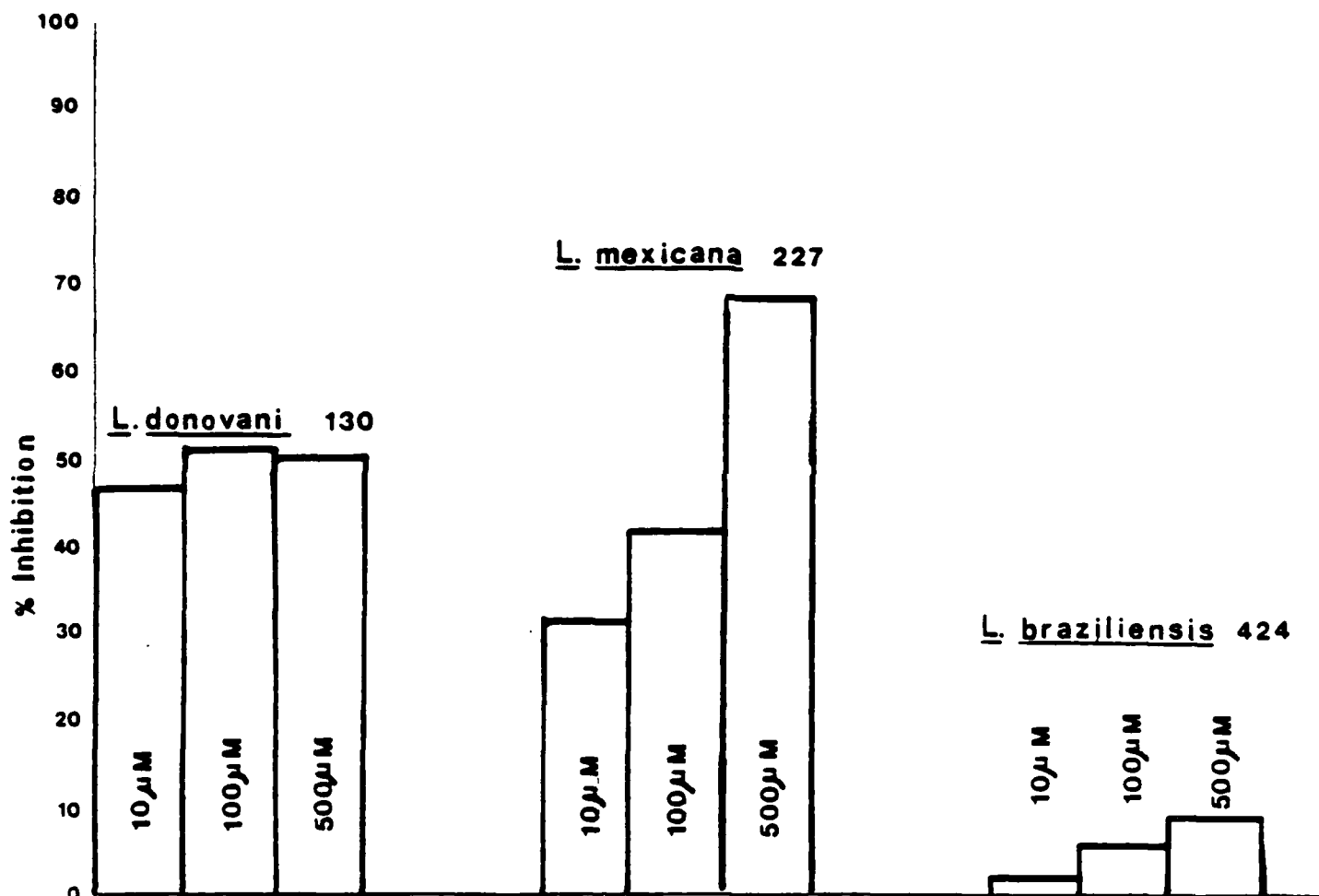
Figure 6

Allopurinol riboside

Drug BK86124

Figure 7

6-Aminoallopurinol Riboside

Drug BK48464

Table 2

Reversal of Analog Inhibition by Natural Purines

Purine Analog 50 µM	% Inhibition				
		Purine Added 200 µM			
	Media Only	Adenosine	Guanosine	Inosine	Hypoxanthine
3-β-D-Ribofurano-sylpyrazolo-[4,3-d]pyrimidin-7-thione BK 63005	70.3	.33.6	43.6	29.4	74.5
Oxoformycin B BK 74731	51.7	9.0	25.4	30.0	58.0
Thiopurinol riboside BK 63863	60.5	59.8	51.8	55.9	60.7
Allopurinol riboside BK 86124	29.6	16.6	29.5	16.8	35.6
6-Aminoallopurinol riboside BK 48464	9.9	6.4	18.4	11.3	6.4
6-Aminoimidazo[4,5-C]pyridin-4(5H)-one BK 95730 (3-deazaguanine)	12.7	12.8	None	13.7	8.9

Compound	Concentration Giving 50% Inhibition of Growth of L. mexicana #227 Promastigotes
Sinefungin	.005
Formycin B	0.1
Aphidicollin	2
4'Thioadenosine	3
Oxoformycin A	4
Deoxyaristeromycin	8
5-Deoxy-5(Isobutylthio)-3-Deazaadenosine (deaza-SIBA)	20
Cordycepin	25
9-Deazainosine	40
Oxoformycin B	50
Allopurinol riboside	200

Tables (3-5) and Figures (8-9) show inhibition of these various compounds alone and in combination with others. As can be seen oxoformycin A and B show no additive effect when used in combination. Oxoformycin A is an adenosine analog and oxoformycin B is an xanthosine analog, so it was believed that the toxicity of these compounds would be additive. The fact that oxoformycin B was found to inhibit growth is an important finding, since this compound has been reported to be non-toxic to both eukaryotes and prokaryotes. It is because of this that oxoformycin B and 9-deazainosine (also proposed to be non-toxic) have been combined together and with sinefungin for studies on growth inhibition. Sinefungin is >100X more active in Leishmania than mammalian cells, and hits a "hot" target (one which cannot be easily modified or mutated--DNA polymerase). It appears that sinefungin does not have to be metabolized to be toxic, and it is not incorporated into DNA because of its structure. Combining sinefungin at extremely low levels, 5 - 2.5 nM, with non-toxic compounds which add to the toxicity of sinefungin via another mode of action should provide a safe and rational approach to chemotherapy of leishmaniasis.

Sinefungin is a naturally occurring antifungal antibiotic nucleoside containing an ornithine residue. This compound has been found to display antiparasitic activity against malarial parasites, T. cruzi, and Leishmania polymerase activity from L. mexicana #227. The enzyme preparations we used had been subjected to cell homogenation, centrifugation, and DEAE cellulose chromatography.

Table 6 summarize some of the compounds we have tested and the primary target enzymes affects in Leishmania.

Table 3   Synergy between Oxoformycin A and Oxoformycin B

Compound	Concentration μM	% Inhibition Alone	% Inhibition When Added Together
Oxoformycin A	20μM	78.6	78.0
	30μM	80.9	80.0
	50μM	83.4	82.6
Oxoformycin B	20μM	37.7	
	30μM	46.0	
	50μM	52.8	

% Inhibition determined at 72 hours.

Table 4 — Synergy between Promising Purine Analogs

Compound	Concentration μM	% Inhibition Alone	% Inhibition When Added Together Concentration (1)	Sinefungin (1)	(2)	Oxoformycin
Allopurinol riboside	50	42.6	61.5			
	100	45.8	61.3			
	200	50.4	57.3			
Oxoformycin B	20	43.2				
9-deazainosine	10	35.7		89.4	35.8	59.1
	20	52.6		91.8	48.2	70.4
	40	73.8		93.0	78.3	78.7
	80	85.8		93.8	85.9	87.3
Sinefungin Conc. 1	0.005	66.6				
Conc. 2	0.0025	5.1				
Oxoformycin B(1)	20	43.4				

16

Table 5   Synergy between Promising Purine Analogs

Compound	% Inhibition Alone	% Inhibition with 5 nM Sinefungin	Predicted Inhibition	% Increase in Expected Toxicity
Sinefungin 5nM	19.71	-	-	-
9-Deazainosine 1μM	5.66	19.71	25.66	Less than 5.95
Formycin B 0.1μM	56.71	85.64	76.42	9.22
Oxoformycin 20μM	27.99	80.92	47.70	33.22
Oxoformycin 4μM	66.04	85.53	85.75	Same
Allopurinol riboside 50μM	37.73	61.63	57.44	4.19

% Inhibition was determined at 72 hours.

Fig. 8   INHIBITION OF L. MEXICANA 227 BY PURINE ANALOGS

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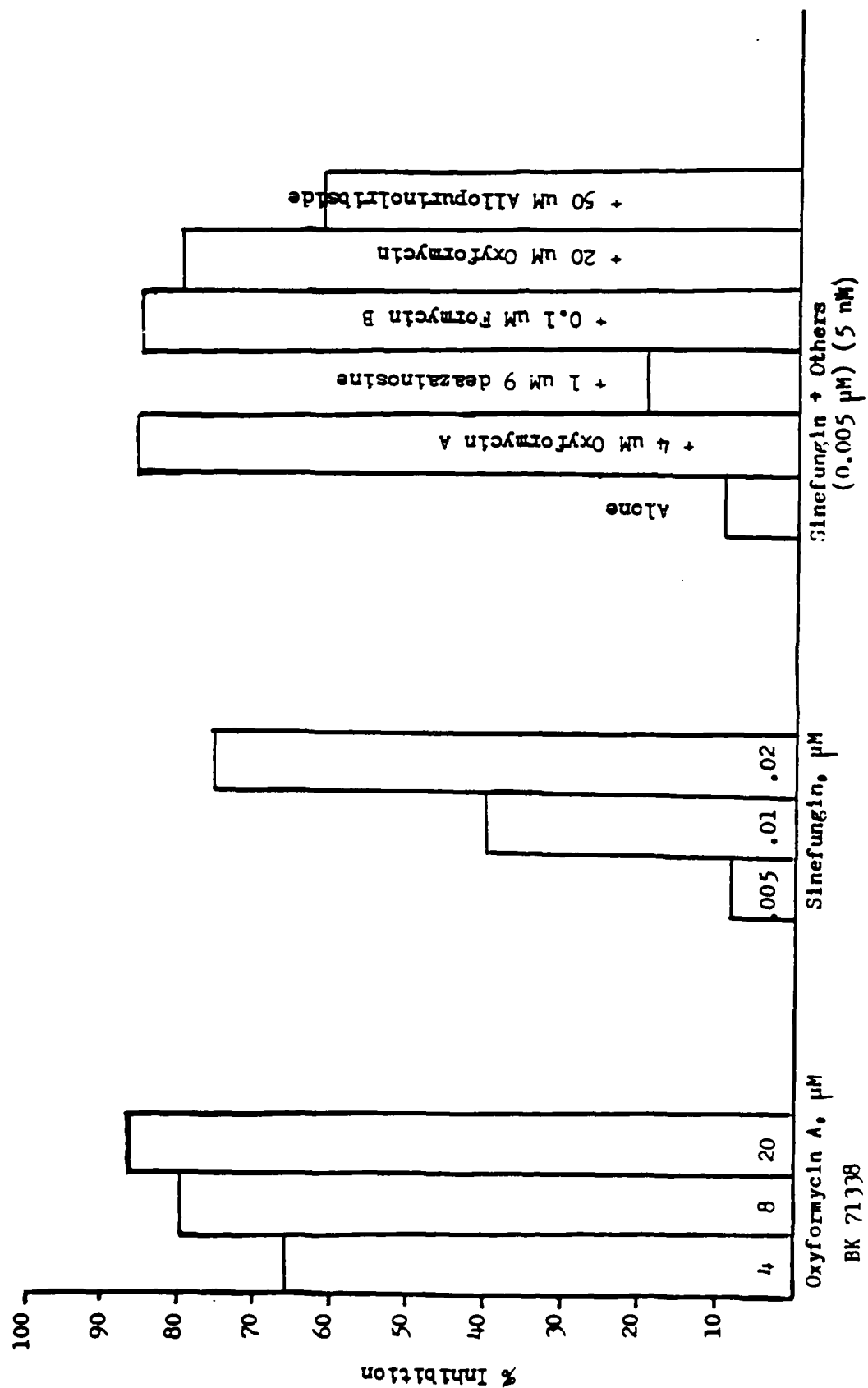
Fig. 9 INHIBITION OF L. MEXICANA 227 by PURINE ANALOGS

Table 6

Potency and Mode of Action of Purine and Pyrimidine Analogs

Compound	Minimum and Maximum Tested	Type of Test, Organism Tested	Maximum Inhibition	Recommended animal Dosage
6-Methylaminopurine-9-ribofuranoside	100 uM	in vivo, L. mex. 227	24%	
Hypoxanthine-9-β-D-arabinofuranoside	100uM	"	36%	
4-Aminopyrazolo(3,4,-d-pyrimidine)	0.5mM	"	91%	
6-Methyloxypurine riboside	0.5mM	"	18%	
6-Mercaptopurine riboside	0.5-1 mM	"	80%	
Adenosine N^6-cyclohexyl	0.5-1 mM	"	66%	
8-Phenyltheophylline	100 uM	"	10%	
Phosphonoacetic Acid	100uM	"	18%	
Ethidium bromide	25uM	"	95%	
Nalidixic Acid	25-100 uM	"	None	
Novobiocin	100uM	"	48%	
Coumermycin (Low solubility)	12.5 uM	"	24%	
Aphidicolin	25uM	"	95%	
6-Mercapto purine	o.5 mM	"	91%	
5-Fluorouracil	0.5 mM	"	82%	
4-Mercapto-2-pyrazolo [3,4-d pyrimidine]	0.2 mM	"	91%	
		in vivo L. don.130	41%	

20

Table 6 (continued)   Potency and Mode of Action of Purine and Pyrimidine Analogs

			Quarterly Report Jan-March, 1984	
Novobiocin	DNA Polymerase	3.5µM		Yes
Coumermycin	"	20µM	"	Yes
Aphidicolin	"	18µM	"	Yes
2-Mercaptopyrimidine	Growth Inhibition	.25mM	#2, pg. 35	No
5-Fluorouracil	Growth Inhibition	.3mM	#2, pg. 35	Yes
4-Mercapto-2-pyrazolo [3,4-d] pyrimidine	Growth Inhibition	.15mM	#2, pg. 35	No

21

DNA Polymerase - We described for the first time the isolation and
characterization of the predominant DNA polymerase from the genus Leishmania
which are parasitic flagellated protozoa. Like mammalian DNA polymerase α, the
leishmanial DNA polymerase is of large molecular weight, is sensitive to
N-ethylmaleimide, and is inhibited by high ionic strength. Unlike mammalian
DNA polymerase α, but similar to the predominate DNA polymerase isolated from
the related organisms Trypanosoma cruzi and Crithidia fasciculata, the
leishmanial DNA polymerase is resistant to inhibition by aphidicolin, a potent
inhibitor of DNA replication in mammalian cells and of DNA polymerase α. The
DNA polymerase α-like was purified over 4,000-fold, and properties such as pH
optimum, salt sensitivity, template requirements and response to DNA polymerase
inhibitors were determined. A DNA polymerase α-like could not be detected
during the isolation procedures.

RNA Polymerase - A DNA-dependent RNA polymerase has been isolated and
characterized from the parasitic flagellated protozoan Leishmania mexicana.
The initial stages of purification utilize high ionic strength extraction and
protamine sulfate treatment. The enzyme was further purified by differential
elution on Heparin-Sepharose, DEAE-Sephadex, and Carboxymethyl-Sephadex
chromatography. Analysis of the chromatographically purified RNA polymerase on
nondenaturing gels revealed two electrophoretic forms. The enzyme isolated has
characteristics of true DNA-dependent RNA polymerase since it requires DNA and
all four nucleoside triphosphates for the synthesis of RNAase-sensitive
products. Analysis of ammonium sulfate and metal ion optima, as well as
relative activities of the enzyme with Mn^{2+} versus Mg^{2+} are similar to
those reported for other RNA polymerase III in eukaryotes.

Formycin A triphosphate was found to be a competitive substrate for this
enzyme, and cordycepin triphosphate was found to be inhibitory, although the
mode of inhibition was not determined.

Summary

On May 17, 1984 a workshop on antileishmanial drug development was set up at WRAIR for the purpose of establishing a major intermural, multidisciplinary drug development research effort. This antileishmanial research program is being directed through the Division of Experimental Therapeutics, and includes three laboratories at WRAIR and three U.S. university laboratories. The role of this laboratory in the program is to test compounds in vivo using different Leishmania spp. and to determine the molecular mode of action of promising compounds. Compounds provided by WRAIR were tested singly, and for synergy, in combination.

The most promising compounds to date sent by WRAIR appear to be the following:

　　　BK63005 3-B-D-Ribofurano-sylpyrazolo-[4,3-d] pyrimidin-7-thione
　　　BK74731 oxoformycin B
　　　BK63863 Thiopurinol riboside
　　　BK71338 oxoformycin A
　　　BK86124 Allopurinol riboside
　　　　　　　9-Deazainosine

The following compounds sent by Dr. Peter K. Chiang (Department of Biochemistry, WRAIR) were found to be very inhibitory to promastigotes of Leishmania mexicana amazonensis WR227 : 4'-thioadenosine, deoxyaristeromycin and 5-deoxy-5 (isobutylthio)-3-deazaadenosine (deaza-SIBA).

Sinefungin, a naturally occurring antifungal nucleoside antibiotic containing an ornithine residue, obtained from Dr. M. Robert-Gero (ICSN-CNRS, Gif Sur Yvette, France) was also found to be very inhibitory.

A DNA-dependent RNA polymerase has been isolated and characterized from Leishmania mexicana WR #227. The initial stages of purification utilized high ionic strength extraction and protamine sulfate treatment. The enzyme was further purified by differential elution on Heparin-Sepharose, DEAE-Sephadex, and Carboxymethyl-Sephadex chromatography. Analysis of the chromatographically purified RNA polymerase on nondenaturing gels revealed two electrophoretic forms. The enzyme isolated has characteristics of true DNA-dependent RNA polymerase since it requires DNA and all four nucleoside triphosphates for the synthesis of RNAase-sensitive products. Analysis of ammonium sulfate and metal ion optima, as well as relative activities of the enzyme with Mn^{2+} versus Mg^{2+} are similar to those reported for other RNA polymerase III in eukaryotes.

Formycin A - triphosphate was found to be a competitive substrate for this enzyme, and cordycepin triphosphate was found to be inhibitory, although the mode of inhibition was not determined.

We have partially purified a DNA polymerase from L. mexicana WR #227 which is N-ethylmaleimide sensitive, aphidicolin resistant and showed different sensitivities to 2-acrylaminopurine deoxyribonucleoside-5'-triphosphate than mammalian DNA polymerase ι. The purification scheme resulted in removal of over 99% of protein with over a 282 - fold increase in specific activity.

MILITARY SIGNIFICANCE

The need for leishmanicides cannot be overemphasized. At present chemo-
therapy is dependent on a relatively small number of synthetic drugs.
Resistance has been reported to occur against all these drugs and devel-
opment of resistance to one compound is often accompanied by cross-
resistance to others. In the chemotherapy of visceral and cutaneous
leishmaniasis, the choice of drugs is very limited and success of a
particular drug appears to vary from locality to locality, presumably due
to strain differences in Leishmania.

To date the logical design of antiparasitic drugs has proved largely
unsuccessful with the exception of purine metabolism in protozoa. While
mammalian cells are capable of de novo synthesis of purines, many para-
sites do not synthesize purines but use salvage pathways. Analogues
inhibiting key enzymes in purine pathway should, therefore, provide novel
therapeutic agents. Purines and pyrimidines serve not only as precursors
of RNA and DNA, but also as stores of high energy phosphate, constituents
of certain coenzymes, and modulators of various enzymatic reactions. In
view of this vital role, intervention of their metabolism will have pro-
found effects on the organism.

To date there is no safe, effective, and quality-controlled anti-
parasitic vaccines. Membrane antigens differ from one species to another
and during the course of infection, making the production of a useful
vaccine very difficult.

The elucidation of the biochemical mode of action of promising compounds
and the identification of unique enzyme systems will permit the logical
design of more effective derivatives and also will provide insight on the
mechanism of drug resistance. This information may allow a therapy pro-
gram to be developed which would decrease or eliminate the problem of
drug resistance.

Targeting of already promising compounds may increase the efficacy of
these compounds for the various disease states of leishmaniasis and be
more cost effective than the development of more than one drug.

Targeting will also allow the reduction in toxicity of certain compounds,
and also be more cost effective since less drug should be required.

24

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